Mouse Modeling for Human Pancreatic Cancer

Gloria Su, Ph.D.

gs2157@columbia.edu, ICRC 10-04

Associate Professor

Departments of Pathology and
Otolaryngology/Head and Neck Surgery
Columbia University College of Physicians & Surgeons

Modeling Human Pancreatic Cancer

- History of cancer
- Pancreatic cancer etiology
- Mouse models for human PC-xenograft, carcinogen-induced, genetically engineered mouse models (GEMMs), 3-D organoids
- Lessons from GEMMs

Cancer History

- DNA structure was proposed by Drs. Francis Crick and James Watson in 1953.
- “Two-hit hypothesis”-multi-mutation theory on cancer-first published by Carl Norden in 1953; popularized by Alfred Knudson in 1971 and also known as “Knudson’s Hypothesis”
- 1971, President Richard Nixon declared war on cancer.
- 1976, Drs. J. Michael Bishop and Harold Varmus discovered the first oncogene (Nobel Prize winners, 1989).
- 1986, Dr. Robert Weinberg discovered the first human tumor-suppressor gene (TSG).
- 1994, Dr. Mark Skolnick linked TSG to familial breast and ovarian cancer, confirmed by Dr. Mary-Claire King the same year.
- 1984, oncogenic KRAS was discovered as an oncogene in lung cancer, and subsequently in PC in 1988.
- 1996, DPC4/SMAD4/SMAD4 was discovered by Dr. Scott Kem.

Epidemiology of Pancreatic Cancer

- Pancreatic cancer is relatively rare (the eleventh most common cancer in respect to incidence). >48,960 Americans are diagnosed with pancreatic cancer annually.
- The 5th leading cause of cancer death due to poor prognosis (~ 40,560 deaths estimated for 2015)- a five-year survival rate is ~7%.
- The highest rates of pancreatic cancer tend to occur in the developed countries.
- The risk factors includes aging, current smoking (OR: 2.20), heavy drinking (>3 drinks/day), obesity (body mass index >30kg/m²), diabetes (>3 years), family history of PC (OR:1.6), non-O ABO genotype, certain SNP alleles, chronic inflammation.
- Pancreatic cancer is a genetic disease.
The pancreas is made up of three cell lineages— islet, acinar, and ductal epithelial cells.>

Pancreatic Cancer

- >75% of human pancreatic cancer are presented as ductal adenocarcinoma of the pancreas that progress from PanIN (pancreatic intraepithelial neoplasm) lesions.
- Others include: mucinous cystic neoplasms (MCNs), intraductal papillary mucinous neoplasms (IPMNs), medullary carcinoma, acinar cell carcinoma, pancreatoblastoma etc.

PanIN (pancreatic intraepithelial neoplasia), IPMN (intraductal papillary mucinous neoplasms), and MCN (mucinous cystic neoplasm) are precancerous lesions of PDAC (pancreatic ductal adenocarcinoma)

Invasive Cancer

- If a tumor is found to be malignant, its extent or spread is measured by a process called staging. The stages of pancreatic cancer are:
  - Stage I: Very small tumors limited to the pancreas (12-14%).
  - Stage II: Larger tumors localized to the pancreas (5-7%).
  - Stage III: The cancer has spread to the lymph nodes, although not necessarily to distant organs (3%).
  - Stage IV: The cancer has metastasized to the colon, spleen, stomach, or more distant organs such as the lungs or liver (1%).
PanIN (pancreatic intraepithelial neoplasia)
- Progresses to pancreatic ductal adenocarcinoma (PDA).
- Microscopic papillary or flat noninvasive.
- Arise from intralobular ducts.
- Columnar-to-cuboidal cells with varying amounts of mucin and degrees of cytologic and architectural atypia.
- Ducts less than 5 mm in diameter.
- Five-year survival following resection is less than 20%.

IPMN (intraductal papillary mucinous neoplasm)
- Progresses to non-invasive or invasive carcinoma.
- Grossly visible, noninvasive, mucin-producing predominantly papillary or rarely flat, epithelial neoplasm.
- Arising from the main pancreatic duct or branch ducts.
- Lesions greater than 1 cm in diameter.
- Five-year survival following resection of IPMN with invasive cancer (43%) or without invasive cancer (77%)
- GNAS, RNF43, STK11, PIK3CA

The Importance of Cancer Genetics Profiling
- Early Detection: High-risk patients (familial and sporadic) and follow-up/recurrence.
- Biomarkers: Prognosis, diagnosis, staging.
- Therapeutic targets
- Chemoprevention
- Animal modeling

Hurdles in PC Research
- Low incidence.
- Most of PC patients die within 6 months of diagnosis.
- Only 15-20% of PC are surgically resectable.
- Resected tumors have lots of normal stromal tissue contamination.
- Early PanIN samples are rare.

Genetics of Cancer
- Tumor suppressor genes: Normally function to restrain cell proliferation, and loss of their activity may lead to unrestrained cell growth (broken brake pedal).
- Oncogene: Encode for protein which, when overexpressed or activated by mutation, possess transforming properties (gas pedal stuck in the "on" position).
- DNA Mismatch Repair Gene: Check the fidelity of DNA replication. When inactivated, errors which normally occur during DNA replication are not corrected (Drunk mechanic).

Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)

- Mutations in genes:
  - *KRAS* (51%)
  - *ARID1A* (45%)
  - *IDH1* (7%)
  - *CDKN2A* (5%)
  - *TERT* (3%)
  - *TP53* (3%)

- GNAS, RNF43, STK11, PIK3CA

The Genetics of Cancer (Maitra et al, Modern Path 16:902-12, 2003)
Global genomic analysis of pancreatic cancer
(Jones et al, Science 2008)
- 24 pancreatic cancer, 23,219 transcripts, 20,661 genes, $\approx 10^6$
  - SNP-pancreatic cancer harbors 63 alterations on average, majority are point mutations.

Global genomic analyses of pancreatic cancer
- 63 alterations can be defined by 12 core signaling pathways.
- KRAS, TP53, SMAD4, CDKN2A, ARID1A, ROBO2 were confirmed and candidate drivers KDM6A and PREX2 were identified.

Majority of mutations occur before metastasis
- Sequencing the genomes of 7 pancreatic metastases.
- A total of 426 somatic mutations in 388 different genes were identified among ~221 millions base pairs sequenced, corresponding to an average of 61 mutations per index metastatic lesion (range 41-77).
- Two categories of mutations were: ‘founder’ mutations (mutations present in all samples from a given patient; mean of 64%, range 48-83% of all mutations per patient). All other mutations were characterized as ‘progressor’ mutations.
- Parental, non-metastatic clones from primary tumors give rise to distal metastatic clonal populations.

Late diagnosis, not its intrinsic aggressiveness, causes high mortality in PC patients
(Yachida et al, Nature 2010; Yachida et al, Oncogene 2013)
- Using Ki-67 as a marker to calculate cell doubling time, as well as the accumulation of passenger mutations to estimate number of passages, it was deduced that it takes at least a decade from the initiating mutation to the birth of a parental non-metastatic clone.
- At least five more years are required for the acquisition of metastatic ability and patients die an average of two years thereafter.

Pancreatic cancer leave home early?
- Tagged pancreatic cells invaded and entered bloodstream early, before frank malignancy could be detected (Rhim et al, Cancer Cell 2012).
- Circulating tumor cells (CTC) were captured in 7/21 (33%) patients with cystic lesions and no clinical signs of cancer, 8/11 (73%) with PDA, and 0/19 (0%) of control (Rhim et al, Gastroentero 2014).

Desmoplasia-Friend or foe?
- Current model: The stroma is protective and is exerted at the PanIN stage (Ozdemir et al, Cancer Cell 2014, Rhim et al, Cancer Cell 2014).
Hurdles in PC Patient Care

- Low incidence.
- Most of PC patients die within 6 months of diagnosis.
- Only 15-20% of PC are surgically resectable.
- Resected tumors have lots of normal stromal tissue contamination.
- Early PanIN samples are rare.
- Complexity of mutations
- Desmoplasia complication
- Metastasis in most patients

The Needs in PC Patient Care

- Early Detection-High-risk patients (familial and sporadic) and follow-up/recurrence.
- Biomarkers-Prognosis, diagnosis, staging.
- Therapeutic targets
- Chemoprevention
- Animal modeling

Generating Models for PC

- Xenografts (human PC cells into mice)
  - Subcutaneous
  - Orthotopic
  - Patient-derived xenografts
- Carcinogen administration
  - BOP into hamsters
  - DMBA into mice & rats
  - Azaserine into rats
- Genetic Engineering
  - Oncogenes: mutant Kras, TGFalpha, SHH
  - TSG: p16, Smad4, p53, TGFbRII, Stk11, etc.
- 3-D organoids

Subcutaneous Xenograft

Implantation of PC from cell lines or resected tissue.

Mouse Modeling for Human Pancreatic Cancer

- A model that recapitulates its human counterpart in tumorigenesis (in both histological progression and genetic mutations).
- A model that allows spontaneous tumor development and yet with predictable time line.
- A model that has an intact microenvironment and yet allows metastasis.
Orthotopic: Implantation into the pancreas

Tsuji et al, J Pancreas 2006; 7:193-9

Carcinogen induced

Tsuji et al, J Pancreas 2006; 7:193-9

Patient-derived xenografts

Tentler, J. J. et al. (2012) Patient-derived tumour xenografts as models for oncology drug development

Mouse Models for PC

- Xenografts (human PC cells into mice)
  - Subcutaneous
    - Pro: Easy & cheap, short-term, high penetrance, easy to quantify tumor burden.
    - Con: No metastasis, no PanIN, lacks intact TME.
  - Orthotopic
    - Pro: Metastasis & short-term, PanIN in some, high penetrance, better mimicking human PDAC histologically.
    - Con: Labor intensive, PanIN in some, more mouse-to-mouse variability, more difficult to quantify tumor burden, lacks intact TME.
  - Patient-derived xenografts
    - Pro: Same as other xenografts plus the potential for personalized medicine.
    - Con: Same as other xenografts

- Carcinogen administration
  - BOP into hamsters, DMBA into mice & rats, Azaserine into rats
    - Pro: Easy & cheap, PanIN in some models, simulate environmental assaults, intact TME.
    - Con: Unknown genetic profile, difficult to monitor progression, few carcinogens have been studied in mice.

Genetically-Engineered Mouse Models

- Strategies
  - Transgenics
  - Knock-in
  - Knock-out

- Targeted genes
  - Oncogenes: mutant Kras, TGFalpha, SHH, GNAS
  - TSG: p16, SMAD4, p53, TGFbRII, STK11, etc.

- Targeted cell types
  - Pancreatic progenitor cells
  - Acinar cells
  - Ductal epithelial cells
  - Centroacinar cells

- Pro & Con
  - Pro: Best mimicking human PC at genetic & histologic levels, intact TME, PanIN development, allows pathway analyses, progression to PDA and metastasis.
  - Con: Expensive, time-consuming, labor-intensive, requires extensive knowledge on gene targeting, may have limited tumor complexity, may harbor secondary (not engineered) mutations.

Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Location (bp)</th>
<th>Mutations (%)</th>
<th>Mutation Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>16036</td>
<td>&gt;90</td>
<td>som.</td>
</tr>
<tr>
<td>p16</td>
<td>9q21.33</td>
<td>26-75</td>
<td>som.</td>
</tr>
<tr>
<td>SMAD4</td>
<td>10q26.1</td>
<td>51</td>
<td>som.</td>
</tr>
<tr>
<td>Kras</td>
<td>12q13</td>
<td>&lt;12</td>
<td>som. &gt; genome.</td>
</tr>
<tr>
<td>BRCA2</td>
<td>13q15</td>
<td>&gt;10</td>
<td>genome &gt; som.</td>
</tr>
<tr>
<td>GNAS</td>
<td>8q22.32</td>
<td>5</td>
<td>som.</td>
</tr>
<tr>
<td>STK11</td>
<td>19p13.3</td>
<td>NLS (95%)</td>
<td>genome &gt; som.</td>
</tr>
<tr>
<td>AKT3</td>
<td>17q21</td>
<td>3</td>
<td>som.</td>
</tr>
<tr>
<td>NFE2L2</td>
<td>2p23.3</td>
<td>3</td>
<td>som.</td>
</tr>
<tr>
<td>SMAD4</td>
<td>16q12</td>
<td>1</td>
<td>som.</td>
</tr>
<tr>
<td>SMAD4</td>
<td>16q12</td>
<td>3</td>
<td>som. &gt; genome.</td>
</tr>
<tr>
<td>BRCA2</td>
<td>13q15</td>
<td>3</td>
<td>som. &gt; genome.</td>
</tr>
</tbody>
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Tumor Suppression/Gene Maintenance Genes

- LSL-KrasG12D; Pdx1-Cre and LSL-KrasG12D; p48-Cre oncomice
  - The first mouse model that develops PanINs that simulate human precursor lesions.
  - It utilizes both knock-in and conditional activation technologies.
  - Conditional activation of KrasG12D at physiological level.
  - The phenotypes of LSL-KrasG12D; Pdx1-Cre and LSL-KrasG12D; p48-Cre are very similar.


### Mutational Profile of Pancreatic ductal adenocarcinoma (PDA)

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<th>Gene</th>
<th>Chromosome</th>
<th>Frequency (%)</th>
<th>Mutation type</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>12p13</td>
<td>60-70</td>
<td>Wildtype</td>
</tr>
<tr>
<td>AKT2</td>
<td>18q11.2</td>
<td>16-20</td>
<td>Wildtype</td>
</tr>
<tr>
<td>MET</td>
<td>7q31</td>
<td>10</td>
<td>Wildtype</td>
</tr>
</tbody>
</table>

**Tumor Suppressor Genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Frequency (%)</th>
<th>Mutation type</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN</td>
<td>10q23</td>
<td>&lt; 50%</td>
<td>Wildtype</td>
</tr>
<tr>
<td>SMAD4</td>
<td>18q21.3</td>
<td>22%</td>
<td>Wildtype</td>
</tr>
<tr>
<td>MDM2</td>
<td>13q14</td>
<td>&gt; 90%</td>
<td>Wildtype</td>
</tr>
</tbody>
</table>

**Mouse Model #1**

**PKP GEMM**

\[ p16^{flox/flox};LSL-Kras^{G12D};Pdx1-Cre \]

**Mouse Model #2**

**AKP GEMM**

\[ Acvr1b^{flox/flox};LSL-Kras^{G12D};Pdx1-Cre \]

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**Pdx1-Cre;LSL-Kras^{G12D}; p53^{R172H/+}** mice have accelerated tumor progression, but the same histology-PanIN to PDA

(Hingorani et al, Cancer Cell 2005)

**Pdx1-Cre; LSL-Kras^{G12D}; Smad4^{-/-}** mice preferentially develop mucinous cystic lesions in the pancreases (MCN vs IPMN)

(Bardessy et al., Can Dev 2006; Izeradjene et al, Cancer Cell 2007)
The Needs in PC Patient Care
- Early Detection: High-risk patients (familial and sporadic) and follow-up/recurrence.
- Biomarkers: Prognosis, diagnosis, staging.
- Therapeutic targets
- Chemoprevention
- Animal modeling

The Utilities of GEMMs
- Tumorigenesis
- Cancer stem cells/tumor initiating niche.
- Early Detection/Biomarkers: Prognosis, diagnosis, staging.
- Metastasis
- Therapeutic targets/Chemoprevention

3-D Organoids
- Acinar to ductal metaplasia
- Primary pancreatic duct epithelial cell cultures

Pro & Con
- Pro: Allows personalized medicine, duplicates PanIN and PDA histology and genetics, bypassing the question of cell origin.
- Con: Lacks TME (if xenografted), require transforming growth factor β (TGF-β) pathway inhibitors (A83-01 and Noggin), R-Spondin1 and Wnt/β-catenin-conditioned media, EGF, and PGE2 for propagation; potential issue of cell origin.

Future Directions
- Early Detection
  - Exosomes
  - Circulating tumor DNA (ctDNA)
  - Circulating tumor cells (CTCs)
- Combination therapies
  - FOLFIRINOX
  - Nab-paclitaxel (Abraxane) plus Gemzar
  - Other target therapies (angiogenesis, metabolism, KRAS & other oncogenes, immunotherapies, autophagy, etc)
- Metastasis
  - Tumorigenesis
  - Clonal expansion
  - Treatment options
- Cell Origin
  - PanIN vs. IPMN
  - Ductal vs. Acinar